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Comparison of Segmentation Methods on Brightfield Image Dataset BF-C2DL-HSC

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Abstract

Compared with fluorescent microscopy, bright field microscopes could avoid the potential toxic effect of dyes and allow samples to be put back for further cultivation. However, the low contrast of bright field images has made Image segmentation and cell tracking quite challenging. In this work, the segmentation effects of four methods on bright field image dataset BF-C2DL-HSC are explored. Results showed that the algorithm involving python function "clear_border" works well in eliminating the background interference and accurately labelling cells. The segmentation results produced by this work could be used directly for detecting cell number growth and tracking the movement of these cells.

Keywords

Bright field images; image segmentation; classical methods; Unet.

1. INTRODUCTION

Cell migration and proliferation are two important processes in normal tissue development and disease [1]. Monitoring the proliferation rate and migration pattern of special cell groups, such as stem cells and cancer cells, will be meaningful for relevant researches and drug discovery [2]. The advent of new live cell imaging techniques have made it possible to generate large image datasets [3]. While this can provide abundant information for experiments on cell behaviors [4], it also imposes additional challenges for image segmentation and cell tracking.

Cell Tracking Challenge is a competition under the auspices of IEEE Inter- national Symposium on Biomedical Imaging, which evaluates the performance of algorithms submitted by participants on image segmentation and cell tracking of selected datasets [2]. The dataset BF-C2DL-HSC, used for cell segmentation and tracking in this paper, is a newly added 2D+ dataset in Cell Tracking Challenge. It is from the Baxter laboratory for stem cell biology in Stanford University and contains bright field images of mouse hematopoietic stem cells in hydrogel microwells.

By analyzing the algorithms developed by previous participants, it is known that neural network combined with watershed algorithm works well on several old datasets for segmentation. However, this method does not work as well as expected on our dataset, which features small and densely packed cells. Moreover, the background interference of microwells and the low contrast of bright field imaging also add to the difficulty of segmenting these images. Based on the these problems, we intend to use four traditional algorithms and a neural network algorithm for cell segmentation, and compare the results to find the most suitable segmentation

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algorithm for this dataset. By comparing and improving the performance of several segmentation methods, we have finally figured out one which could successfully eliminate the background interference of microwells and accurately label cells in most images.

2. RELATED WORK

2.1. Introduction of Unet

The emergence of CNN makes many image classification problems easier. CNN completes the feature extraction and classification from images by designing convolution layers, pooling layers and full connection layers. CNN can be used not only to classify the whole content of an image, but also realize image segmentation to determine which label each pixel in the image belongs to. In order to classify a pixel, an image block around the pixel is used as input of CNN for training. Obviously, CNN has opened up a new way to extract complex image segmentation. The applicability and robustness of this model are very good. In most cases, people only need to provide different types of label image training model to achieve the goal, instead of searching for the characteristics of a certain kind of objects and designing complex algorithms and conditions for image segmentation. However, CNN has two obvious disadvantages in image segmentation. First, there will be a lot of redundant computation in convolution, because the pixel blocks of adjacent pixels will have many repetitions. These repeated works waste the storage space and reduce the calculation efficiency. Secondly, because the size of the pixel block is set artificially, but the size of the object to be recognized in the image is different, so the size of the sensing area also limits the performance of pixel classification. On the basis of CNN, Fully convolutional networks (FCN) transforms the full connection layers into convolution layers. By means of deconvolution, FCN restores the abstract features to the image to complete image segmentation. There are two advantages of FCN, one is that it can accept any size of input, the other one is that the segmentation based on image features can be integrated into the neural network. UNET redesigns the network model based on FCN [5]. In the process of upsampling, not only the information from the former layers, but also some features from the shallow alluvium will be considered. This enables the UNET model to consider both abstract feature information and simple graphic feature information for image segmentation. UNET is also very suitable for segmenta- tion of cell images with different characteristics. The disadvantage is that there is some space to improve the segmentation accuracy. For example, in data set Fluo-N2DL-Hela, nucleus and cell will be confused. In data set BF-C2DL-HSC, it is difficult to segment the boundary of closely arranged small cells.

2.2. Segmentation Algorithms

The segmentation algorithms submitted by previous participants can be broadly classified into deep learning methods and classical methods. Most of those methods are publicly available through the website (http://celltrackingchallenge. net/) [3].

In general, deep learning methods use convolutional neural networks (CNN) to generate binary masks, predicting seed pixels for further segmentation by algorithms such as watershed. In most cases, applying deep learning methods could contribute to the performance of segmentation algorithms on datasets, probably because it uses human-annotated images directly to train the network and the iterative learning process allows parameters optimization as the number of epoch increases. For example, the algorithm named CVUT-CZ incorporates a Unet-inspired CNN, J-net, to predict for each pixel its probability of being part of a cell (segmentation) and its distance to the nearest cell boundary. Then seeded clustering is used to partition blobs into different cells. This algorithm performs well, especially for Fluo-N2DL-Hela and phC-C2DH-0373.Yet as images in BF-C2DL-HSC feature smaller cells and low contrast, CNN fails to yield satisfactory thresholding results.

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Another well-performed algorithm called KTH-SE, on the other hand, re- lies on simple thresholding for segmentation. bandpass filtering, ridge detection, template matching and intensity variance are used respectively for several datasets to generate binary masks, followed by seeded watershed to break mul- tiple cells into individual regions. David N. Mashburn [4] has also shown that the watershed algorithm has the flexibility to use a different number of seeds for each cell. And it has user-selectable parameters, which means that users do not need previous image processing expertise to guide parameter selection. In MPI-GE algorithm, the challengers use CNN to detect the center of mass, and then use watershed algorithm to segment cells.

Inspired by these algorithms, watershed segmentation is applied after thresholding by otsu method and CNN respectively. However, segmentation results suffer severely from the problem of oversegmentation, with the number of labelled objects exceeding the number of cells to a large extent. In other classical methods, ISODATA algorithm can effectively segment grayscale image. However, Xiaodong Yang [2]and his team show that it cannot separate touching objects. Because there are a large number of touching cells in BF-C2DL-HSC dataset, this method is not considered for cell segmentation.

Among all the challengers, few of them use clustering algorithm or superpixel algorithm alone to segment cells. In all clustering algorithms, kmeans algorithm is a common one, which can quickly segment the cells in the image by category and needs less parameters to be adjusted. One of the superpixel algorithm called SLIC combines the advantages of kmean algorithm, and uses the mean clustering method to generate superpixels efficiently, which gets cell boundary clearly and segment cells better [6].

Inspired by above algorithms, kmeans clustering algorithm and SLIC algorithm are used for cell segmentation, in order to compare the results of classical algorithms when doing cell segmentation. However, because some parameters in these algorithms need to be adjusted manually, it is very time-consuming to process thousands of images in our dataset. And although the segmentation images can get a clear cell boundary, it cannot label the correct cell number. This problem is related to the background interference of microplates.

3. METHODOLOGY

For segmentation, the performance of four methods on dataset BF- C2DL-HSC are compared. For classical methods, 49 images from training 01, along with the human-annotated segmentation images from SEG, are used to provide reference for parameter adjustment in classical methods. Then 35 images from challenge 01 selected with equal interval between t0063 and t1763 are used to verify the effectiveness of these methods. For Unet,100 images selected between t1264 and t1754 with equal interval, along with the corresponding ground truth from TRA, are used to train the neural network. Then all the images in training 01 are segmented to test the neural network. The detail of these segmentation methods are described as follows.

Unet-based watershed method. There are several steps to complete cell segmentation with U-net model. First, the cell label image in the data set is processed by morphological expansion. Secondly, the image is cropped and zoomed out. Third, build the U-net model and complete the training work. In this process, the Adam optimizer, focal loss function, is used for training. Focal loss function [7] is the formula 1.

$$\text{Loss} = -\text{alpha} * ((1 - p)\text{gamma}) * \log(p) \quad (1)$$

Alpha means the same as wight factor in balanced cross entropy. Gamma means focusing parameter for modulating factor (1-p).

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The model has three advantages. First, it can overcome the change of cell shape and complete rough cell recognition. The image of this dataset comes from a set of sequential images recorded by a microscope. In these images, the cell will appear some changes such as position movement, cell membrane shape changes, mitosis and so on. U-net model can overcome these difficulties and recognize the approximate space occupied by cells in the image. Second, it uses focal loss function in back propagation. This is an unbalanced data set, because most of the time, especially in the beginning, the cell area is limited. I use focal loss function instead of cross entropy loss function. Thirdly, the training speed of this model is also very fast. The experiment shows that the training can be basically completed in 10 epochs. This will be explained in detail in the later experimental results section.

This method also has some disadvantages. In the face of a dense array of cells, it sometimes fails to make a good segmentation of different cells. Secondly, in some data sets, it is difficult for him to distinguish cell boundaries clearly. Because sometimes, the cell boundary is covered with black bubbles, but this is not part of the cell. This does not automatically adapt to these changes. Therefore, after the initial segmentation of UNET, we need to use the traditional algorithm to segment the details accurately.

Here, instead of completing the whole segmentation part, U-net only splits foreground and background pixels, generating binary masks for further seg- mentation by watershed method. The watershed algorithm is an image region segmentation method. In the segmentation process, it uses the similarity between adjacent pixels as an important reference basis, so that pixels that are close in spatial position and have similar gray values are connected to form a closed contour. In our experiment, the markers for watershed segmentation are generated by labelling the local maximum based on distance transform matrix, and binary masks are provided by U-net.

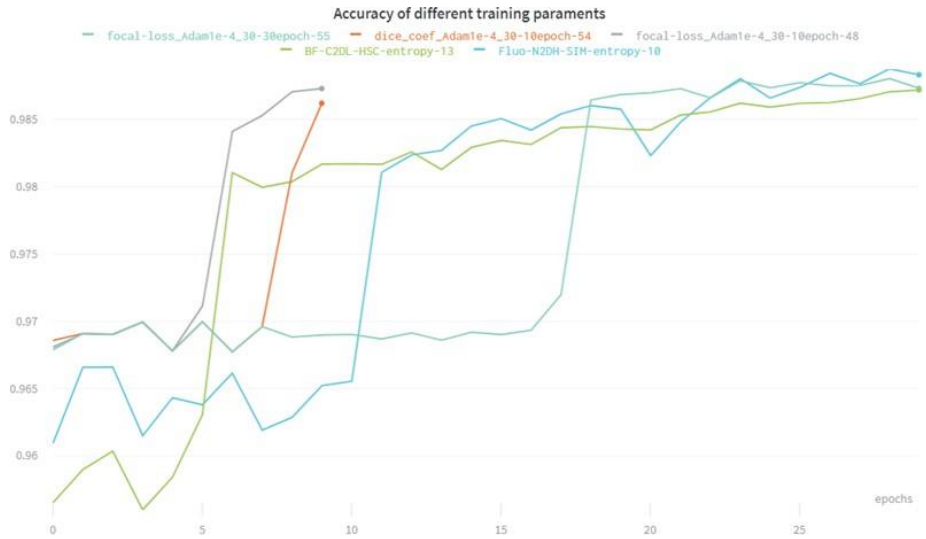

Figure 1. Accuracy of different training parameters.

Initially when using cross entropy loss function, U-net fails to identify cells in BF-C2DL-HSC while working normally on dataset Fluo-N2DH-SIM. This is probably because cells in images from BF-C2DL-HSC only occupy a small proportion of the whole image area. Using Focal loss function for back propagation could increase the weight of cell regions and thus solve this problem of unbalanced dataset.

Fig 1 shows the change of accuracy in different parameters of training. It can be seen that the focal loss function converges faster. Normally no more than 10 epochs can complete the training.

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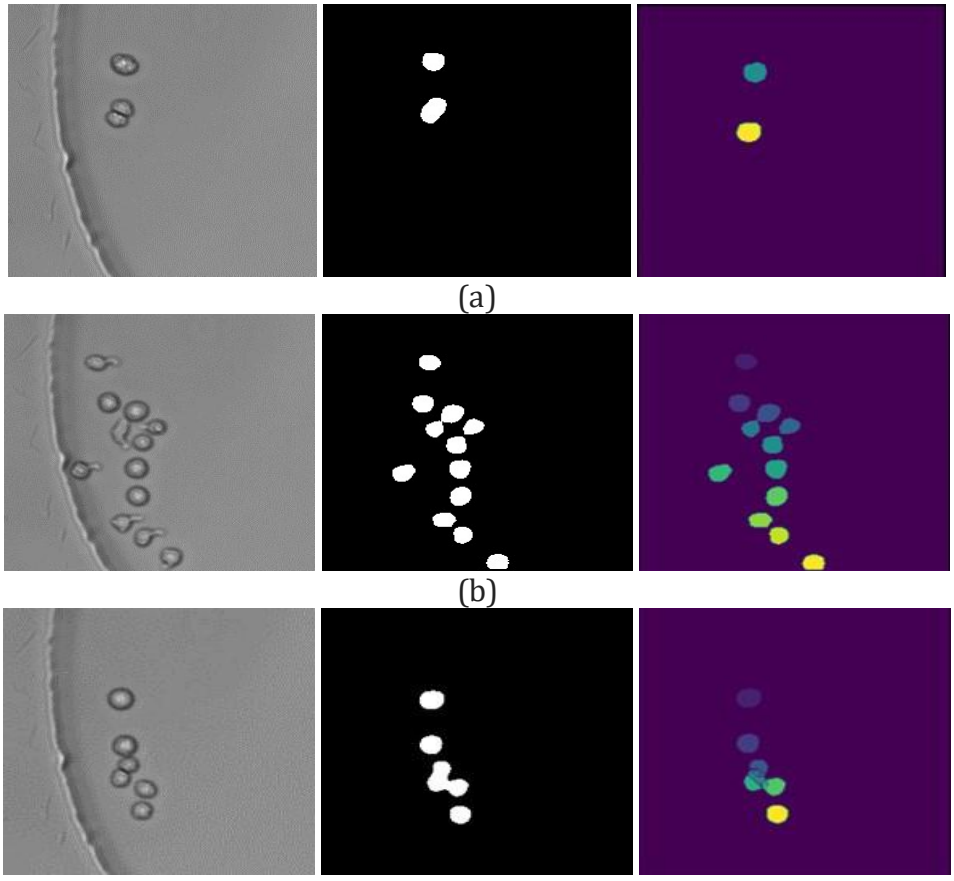
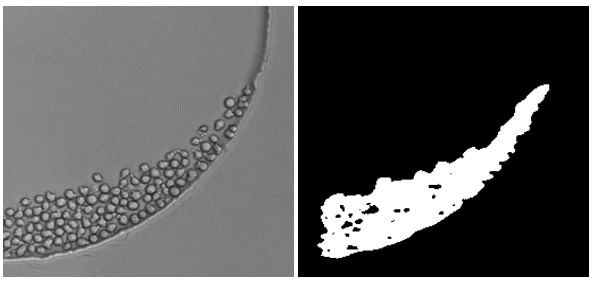


(a) (b) (c)

Figure 2. Image segmentation by Unet-based watershed algorithm. (a) image t632: two cells are not separated. (b)image t1764: all cells are accurately labelled. (c)image t1138:cells are oversegmented.

In general, this method works effectively in identifying cell regions, although occasionally several closely located cells are not separated into individuals (see figure 2a), and sometimes oversegmentation occurs, generating several labels for a single cell (see figure 2c). A major problem with this method is that the Unet fails to separate densely packed cells. While this disadvantage does not affect the segmentation results of training 01, in which none of the images have more than 12 cells, it restricts the application of CNN on challenge data, as shown in figure 3.



(a) (b)

Figure 3. (a)original image from challenge dataset (b)segmentation result by Unet

Clear border-based method. Images are cropped and preprocessed by me- dian filter in the first place. Threshold for binary image is selected as the minimum of 0.5 and the one determined by otsu method. Morphological erosion and dilation by 5x5 square are performed

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to avoid the problem of missing cells caused by unclosed cell boundaries. Then the python function clear border, which works to clear pixels with value 1 on the border of a binary image, is applied to eliminate the background interference of microwells.

In most cases, directly labelling the images processed by clear border would lead to accurate detection results. While sometimes additional steps, which involve removing labels with low intensity value or large areas, are needed to clear up the background pixels (see figure 4b). Occasionally, one or two cells might be over-eroded and thus ignored as a cost for closing cell boundaries (see figure 4c).

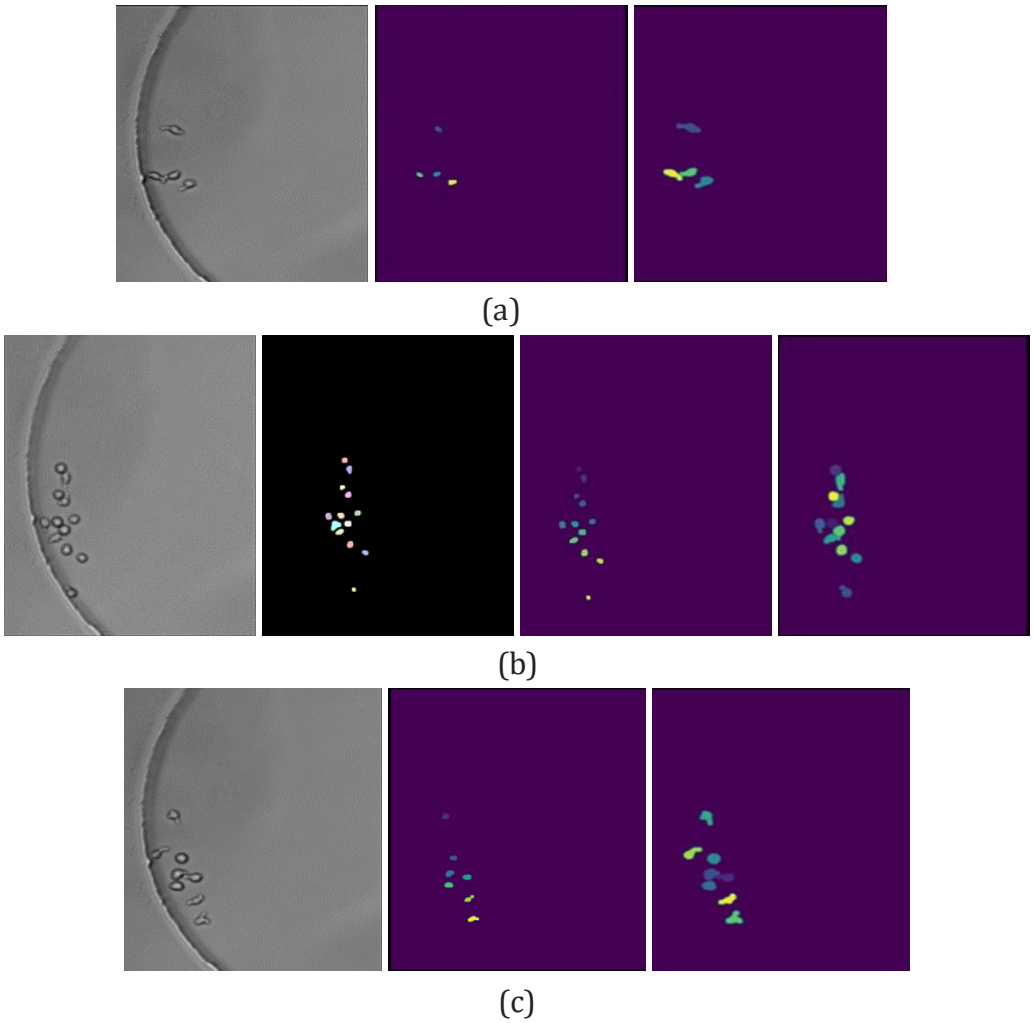

Figure 4. Image segmentation by clear border-based method. In each row, the first and the last image are original image and ground truth respectively. (a)image t932: all cells are accurately labelled. (b)image t1707: the second picture shows how segmentation results are influenced by background noise; the the third image is obtained by removing labels with area >150 from the second image. (c)image t1460:one cell is fully eroded and thus unlabelled.

Canny edge detector/Sobel operator with Kmeans method. The median filter is used to denoise the original images first, and then each image is processed by Canny edge detector and Sobel operator respectively to observe the influence of different preprocessing methods on the kmeans algorithm. The standard deviation of Gaussian filter sets as 2 in Canny edge detector. After that, the image is cut uniformly. Because the number of clusters in the kmeans algorithm needs to be adjusted manually, the clusters of all the pictures pro- cessed by Canny edge detector are 2 and the clusters of the pictures processed by the Sobel operator are 3 in order to

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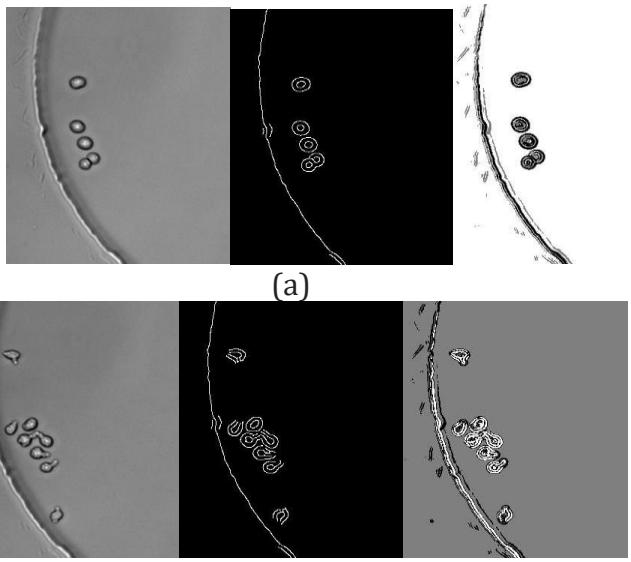
ensure the uniformity of image processing. Both two methods operate this algorithm 1 time, which means the parameter 'init' equals 1. After thresholding by OTUS to reduce background interference, measure.label function in python is used to count the marked cells.



(a)

(b)

Figure 5. Image segmentation by Canny edge detector/Sobel operator with Kmeans method. (a) picture t1122 processed by Kmeans method (b) picture t1471 processed by Kmeans method. For the third column, there are different background colors when using sobel operator with Kmeans.

The images preprocessed with both methods can get clear cell boundaries, but the images processed with Kmeans after canny are the same as those processed with canny only. This may be related to the fact that the image after canny processing only has two colors, so clustering cannot be performed when clusters are greater than 2. However, the background colors of the images processed by Sobel is different, which is related to, we conjecture, the different gray value of each image after Sobel (see Figure 5). The label results of the two methods are not very accurate, but the images processed by canny edge detector is better. Sometimes cells in the images processed by Sobel can not be detected.

Two filters/Unet with Superpixels method. Before using superpixels, two filters, Gaussian filter and median filter, are used to denoise the original images, and then cut the images having cells. Superpixels algorithm cannot directly process the array values of the images denoised by the two filters, so the data in the array needs to be changed to a value between - 1 and 1, and then these float images are converted to uint8 images. Then the superpixels algorithm is used to segment the image. The segments parameter is 1500, the parameter of balances color proximity and space proximity is 0.1, and width of gaussian smoothing kernel for pre-processing for each is 0.5. After that, normalized cuts are used to display the image by clustering, and finally measure. label function is applied to calculate the number of labeled cells.

Another method is to use the images which has been preliminarily segmented by the Unet, and then use the superpixles algorithm to segment the image again. Because in the image processed by Unet, the cells next to each other will have the problem of cell adhesion, which will make the counting cells become a difficult problem, so it is expected that the superpixels algorithm could segment these small cells as much as possible. In this method, the segments parameter is 1100, and it does not need to adjust the array values of images. The other parameters and processing procedures are the same as the previous one.

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The first method which only uses classical algorithms, the segmentation results of the two filters are similar. In this method, the number of labelling cells is inaccurate, but the gaussian filter method can better remove the influence of background microwells on segmentation results (see Figure 6). In the Unet method, there is no noise of the background, but the problem is oversegmentation will appear and a complete cell can not be detected correctly. By changing the segments parameters, it is found that if the parameter value is too small, the connected cells cannot be segmented, but if the parameter value is too large, the oversegmentation problem is very serious.

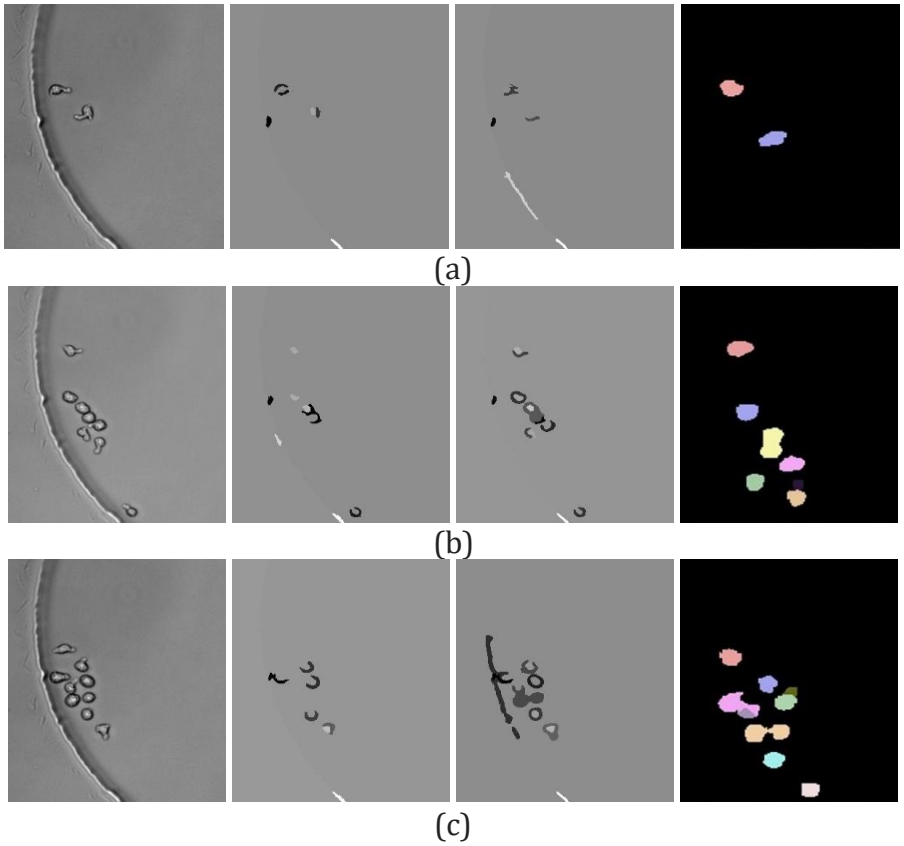

Figure 6. Image segmentation by Two filters/Unet with Superpixels method. (a) picture t402 processed by Superpixles method:the right one is an accurately labelled image using Unet with Superpixles.(b) picture t1490 processed by Superpixles method: Cells cannot be divided when there are lots of adhesions.(c) picture t1583 processed by Superpixles method:the connected cells in right image (Unet) are not completely divided by superpixels, because part of one cell is divided into another.Compared with the second column and the third column, gaussian filter with superpixles has less interference of microwells than median filter with super- pixles.

4. EXPERIMENTAL RESULTS

Experiments are conducted on training 01 and challenge 01 from dataset BF- C2DL-HSC. We will demonstrate the use of four segmentation algorithms on 49 images from training 01 and 35 images from challenge 01, as well as discussing the performance. The best performed algorithm is also applied on all images from challenge 01 and training 01, while the results are not demonstrated here due to the lack of ground truth.

Evaluation metrics For segmentation, the evaluation metrics involve measuring number of labels, Precision and Recall. The number of labels computed by four algorithms will be compared with the human-annotated images, and accuracy is defined as the proportion of

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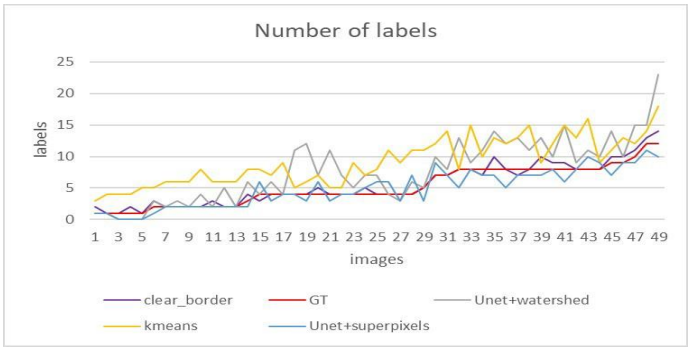
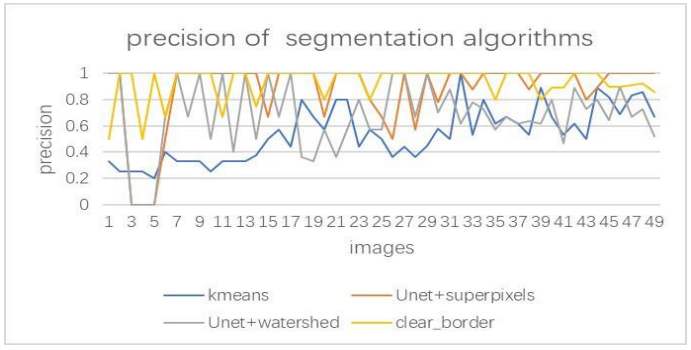
images which have been given correct number of labels. Precision represents the proportion of selected items which are relevant, while Recall measures the percentage of relevant items being selected. These two indices are initially calculated for each image, then the average score of all test images from the same dataset will be used to represent the overall performance of a segmentation algorithm on this dataset. Evaluation results are only provided for 49 images from training 01, for which human-annotated images have been provided.

Figure 7a shows the number of labels computed by those algorithms as well as the ground truth. Fig 4b, c shows the precision and recall of those algorithms. Note that precision could be influenced by oversegmentation as well as back- ground noise. For example, when the only cell in an image is oversegmented and given 2 labels, the value for index precision will be 50%.

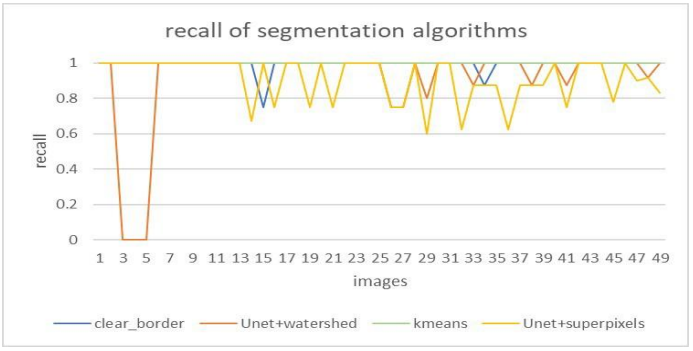
Segmentation methods with clear border have the highest score for all of the Indices. This method works effectively in clearing up the background noise, thus the inaccuracy concerning precision is overwhelmingly caused by oversegmentation. Moreover, by manually adjusting parameters of morphological operations, or removing labels with large areas or low intensity afterwards, performance of this algorithm could be improved as shown in figure 8.



(a)



(b)



(c)

Figure 7. Evaluation results of four segmentation methods. (a) number of labels (b)precision (c)recall

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Nevertheless, when it comes to a whole dataset which contains thousands of images, it is impossible to manually adjusting parameters or taking additional steps for certain images to get better segmentation results. Thus we still use original parameters when dealing with all images of training 01. If manual adjustment allowed, this segmentation methods fail only when the cell boundary of a cell could not be closed until the square area of erosion is large enough to eliminate another cell (see Figure 4c).

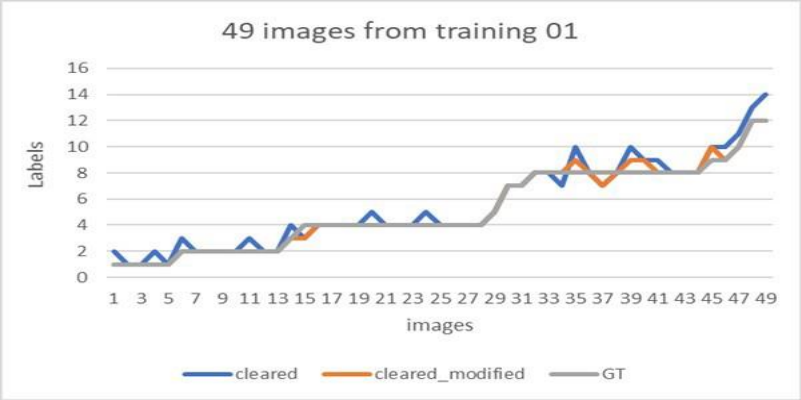

Figure 8. Comparision of segmentation results by parameter adjustment.

Cleared and cleared momdified correspond to original segmentation results and those produced after manually adjusting parameters respectively.GT shows the ground truth. Accuracy increases from 61.2% to 87.8% after parameter adjust- ment.

Although the performance of Unet-based algorithms are not satisfactory from the perspective of these three indices, Unet actually works well in remov- ing the background interference of microwells, despite the fact that some cells are not separated totally. For the first few images without labels, some cells are mistakenly removed during image cropping and rescaling, which is one of the problems we have yet to solve due to the time constraint. Occasionally some cells are ignored or merged due to the low intensity of labels or morphological expansion, which explain why there are several images with recall value 1. For segmentation results produced by watershed algorithm, oversegmentation is a major problem which has severely influenced its scores for precision. however, compared with superpixel algorithm, watershed works better in separating connected cells. For the segmentation results of the superpixels algorithm, the algorithm can not completely segment the connected cells, only a small part of some connected cells can be separated. This may be related to the value of parameters and the feature used in the superpixels algorithm. Because the difference between the number of cells in each image is too large, and the parameters of the superpixels algorithm need to be adjusted manually, it is impossible to find a set of parameters suitable for all images. The feature used by superpixles algorithm is only intensity, and it is speculated that better segmentation results could possibly be obtained when using other features.

Before using the Kmeans algorithm, this paper uses two preprocessing methods. Because the background of the image processed by Sobel operator is different and there is a situation that cells cannot be marked, here discussion is limited to images processed by Canny edge detector, which has a better result. As the images processed by Canny edge detector can only show the edge of the cells, even if Kmenas is used to segment the cells, the cluster parameter, which equals 2, can be used to mark the edge only, not the whole cell. When using Canny edge detector before Kmeans, one cell will detect two or more layers of edges, and then the labeled part of one cell will change from one to two or more. So the accuracy of kmens algorithm is very inaccurate, and the precision is very small.

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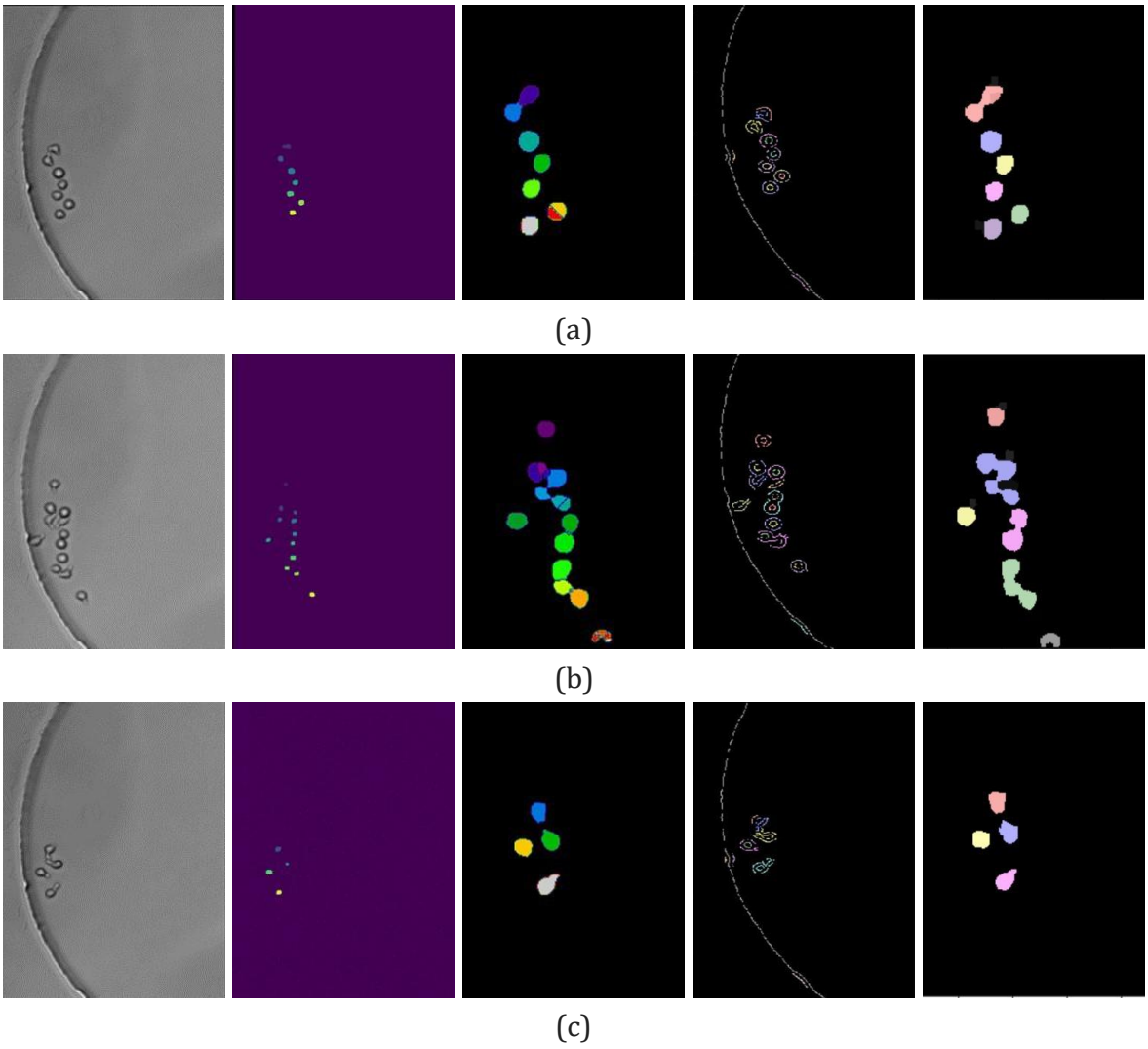

Figure 9. Examples of segmentation results on training 01:From left to right: original images, results of clear border method, re- sults of Unet-based watershed method, results of Kmeans method, results of Unet+superpixels method. (a)image t1170. (b)image t1743. (c)image t731.

Table 1. Average scores of four segmentation methods on training 01

method / metics	Clear_border	Unet+watershed	kmeans	Unet+superpixels
Recall	0.99	0.92	1.00	0.91
Precision	0.93	0.68	0.53	0.87
Accuracy	0.61	0.20	0.02	0.37

Segmentation results on challenge 01 As there is no ground truth image for challenge 01 and the densely packed cells make it difficult to count the labels manually, the performance is only described qualitatively.

Among the four segmentation methods, only clear_border and Kmeans are able to work on challenge dataset, due to the failure of Unet on segmentation of densely packed cells. Clear_border method is still the best-performed one on challenge dataset, while additional

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steps of removing labels with low intensity(intensity<0.5) and large areas (area>300) in order to eliminate the background interference of microwells. These steps may remove several cell labels at the same time, thus the accuracy of this method on challenge 01 is not as good as on training 01.

Similarly, the background of the image processed by the Sobel operator is different, and there is a situation that cells cannot be labeled, so only the results processed by the canny edge detector are discussed here. Kmeans algorithm has the same performance in training dataset and challenge dataset. The results show that the influence of microwells can not be removed, but this algorithm can show the cell edge very clearly. However, a cell will still be labeled many times, so the accuracy of this method could not be determined for the challenge dataset.

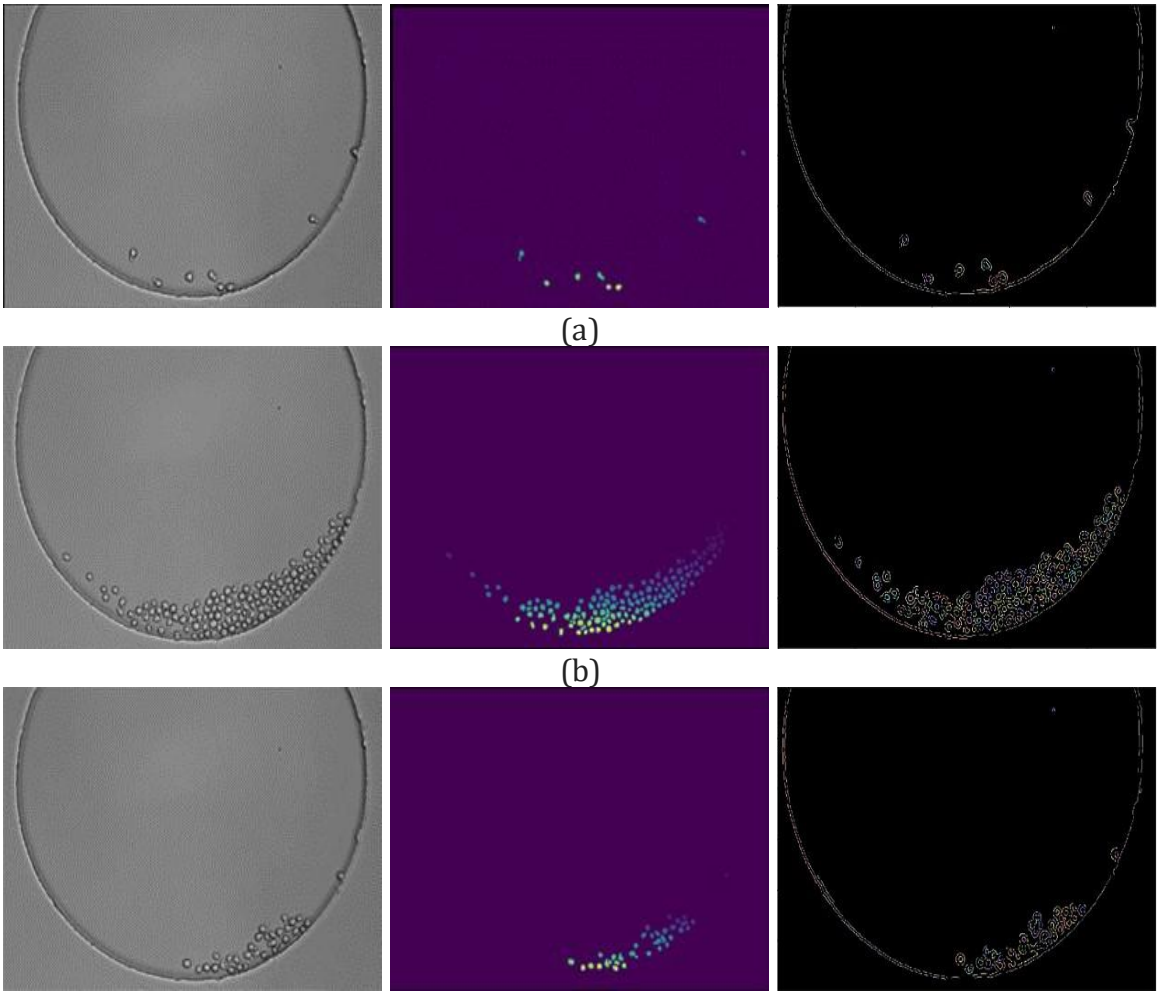

Figure 10. Examples of segmentation results on challenge 01.From left to right:original images, results of clear border method, results of Kmeans method.(a) image t663.(b)image t1713.(c) image t1163.

5. CONCLUSIONS

BF-C2DL-HSC is a newly added bright field images for cell-tracking challenge competition this year. The difficulty of tracking cell movement in this dataset is increased by the low contrast of images along with the strong interference of background microplate. Here in this work ,several segmentation strategies, including both classical methods and deep learning algorithms, are explored to see how these problems could be solved. After comparing the performance of four methods, it is found that the clear_border method combined with steps such as removing low-

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intensity labels works well for cell detection on dataset BF-C2DL-HSC,especially on training 01 ,where the majority of images are accurately labelled and only one or two cells are missing or oversegmented for the rest of the images. Moreover, the neural network Unet has been successfully adapted and applied to this dataset, despite the fact that there is still room for improvement. The results could serve as a foundation for further improvements on segmentation algorithms, pointing out some potential suitable methods worth exploring. Also, the segmentation results of clear_border methods on the whole dataset are accurate enough to be used for the following tracking work, which would also be the focus of future work.

ACKNOWLEDGEMENTS

As all authors contributed almost equally to this work, author names are listed in no particular order.

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